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Part 9112
Revised March 2020

Differential Stain Kit, Smears & Touch Imprints - Technical Memo

KIT INCLUDES:	**Part 9112B**
Solution A: Xanthene Stain	500ml
Solution B: Thiazine Stain	500ml
Solution C: Fixative	500ml

Individual stain solutions may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:
Xylene, ACS Part 1445

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply Differential Stain Kit, a modification of the Wright Giemsa Stain, uses a methanol fixative and aqueous based stains to provide a rapid 3-step process for differential assessment of: peripheral blood smears, touch imprints, fine needle aspirations (FNA), bone marrow biopsy aspirations, and detecting microorganisms.

METHOD:

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply Stain Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

STAINING PROCEDURE:

1. Prepare within an accepted time frame, a well-made blood smear, touch imprint, FNA smear or bone marrow aspiration smear/film per your laboratories protocol, with a focus on uniform cell distribution.
2. Allow slides to thoroughly air-dry prior to staining.
3. Dip dried slides in Solution C: Fixative 5-10 times, one second per dip. Allow excess fixative to drain.
4. Dip in Solution A: Xanthene Stain 5 times, one second per dip. Allow excess solution to drain.
   a. *See Procedure Notes #1, #2 and #3.*
5. Quickly rinse slides with distilled water.
6. Dip slides in Solution B: Thiazine Stain 5 times, one second per dip. Allow excess solution to drain.
7. Rinse slides quickly in distilled water.
8. Allow slides to air-dry, then examine microscopically.
9. If coverslip is preferred, allow slides to air-dry; dip dried slides in xylene and coverslip with compatible mounting medium.

RESULTS:

Erythrocytes:	Pink to yellowish-red
Platelets:	Violet or purple granules

Granulocytes

Neutrophils:	Nucleus - Dark blue to violet Cytoplasm - Pale pink Granules - Purple to lilac
Eosinophils:	Nucleus – Blue Cytoplasm – Blue Granules - Red to red-orange
Basophils:	Nucleus - Purple or dark blue Granules - Dark purple

RESULTS CONTINUED:

Mononuclear Cells

Monocytes:	Nucleus – Violet Cytoplasm - Sky blue
Lymphocytes:	Nucleus – Violet Cytoplasm - Dark blue
Bacteria/microorganisms:	Deep blue in varying shapes
Muscle and collagen	Pale Pink
Nuclei	Blue/violet
Cytoplasm	Varying shades of light blue

PROCEDURE NOTES:

1. The division of stains in this kit gives the user the advantage of varying dips in Solutions A and B to produce different degrees of shading and intensity. However; never use fewer than three dips of one full second each.
2. If more intense stain is desired, increase dips in Solutions A and B.
   a. *To increase eosinophilic staining; increase dips in Solution A.*
   b. *To increase basophilic staining; increase dips in Solution B.*
3. If a paler stain is desired; decrease dips in Solutions A and B.
4. If using a xylene substitute, closely follow the manufacturer's recommendations for coverslipping application.

REFERENCES:

1. Bain, B.J. "Bone Marrow Aspiration". *Journal of Clinical Pathology* 54 (2001): 657-663.
2. Cox, Charles. "Accuracy of Intraoperative Imprint Cytology for Sentinel Lymph Node Evaluation in the Treatment of Breast Carcinoma." *Cancer Cytopathology* 105.1 (2005): 13-20.
3. "Guidelines of the Papanicolaou Society for Fine-Needle Aspiration Procedure and Reporting." *Diagnostic Cytopathology* 17 (1997): 239-247.
4. McPherson, Richard and Matthew Pincus. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. 22nd ed. Philadelphia: Elsevier Saunders, 2011. 522-535.
5. Thompson, Samuel Wisley, and Ronald D. Hunt. *Selected Histochemical and Histopathological Methods*. 2nd ed. Springfield, IL: Thomas, 1966. 756-762.
6. Modifications developed by Newcomer Supply Laboratory.

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Differential Stain, *Helicobacter Pylori sp.* in Tissue Sections – Technical Memo

STAIN SOLUTION:	**500 ml**	**1 Gallon**
Solution A: Xanthene Stain	Part 10521A	Part 10521B
Solution B: Thiazine Stain	Part 10522A	Part 10522B

Additionally Needed:	
Helicobacter sp., Artificial Control Slides	Part 4275
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842

For storage requirements and expiration date refer to individual product labels.

APPLICATION:

Newcomer Supply Differential Stain procedure, a modification of the Wright Giemsa Stain, provides a rapid staining method for demonstration of *Helicobacter pylori sp.* in gastrointestinal tissue sections. Procedures for both monochromatic and polychromatic versions of the Differential Stain are provided.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 4 microns
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

STAINING PROCEDURE:

1. If necessary, heat dry tissue sections/slides in oven.
2. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
    a. *Proceed with either the monochromatic or polychromatic staining method.*

Monochromatic Staining Method: *See Procedure Note #1.*

1. Place slides in Solution B: Thiazine Stain for 1-4 minutes depending upon staining intensity preference.
2. Rinse quickly in distilled water to remove excess stain.
3. Allow slides to air-dry in a vertical position.
4. Dip dried slides in xylene and coverslip with compatible mounting medium.
    a. *See Procedure Note #2.*

RESULTS:

Helicobacter pylori sp.	Dark blue
Collagen and muscle	Blue
Nuclei	Blue
Cytoplasm	Varying shades of light blue

Polychromatic Staining Method: *See Procedure Note #1.*

1. Place slides in Solution A: Xanthene Stain for 3-5 minutes.
2. Drain slides briefly; go directly into Solution B: Thiazine Stain for 1-4 minutes depending upon staining intensity preference.
3. Rinse well in distilled water.
4. Allow slides to air-dry in a vertical position.
5. Dip dried slides in xylene and coverslip with compatible mounting medium.
    a. *See Procedure Note #2.*

RESULTS:

Helicobacter pylori sp.	Dark blue
Collagen and muscle	Pale pink
Nuclei	Blue/violet
Cytoplasm	Varying shades of light blue

PROCEDURE NOTES:

1. The timings are suggested ranges. Optimal staining times will depend upon staining intensity preference.
2. The elimination of dehydration steps is necessary to retain the dark stain of the organism.
3. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and coverslipping steps.

REFERENCES:

1. Potvin, Carol. "A Modified Diff-Quik Stain for *Helicobacter pylori* in Gastrointestinal Biopsies." *Laboratory Medicine* 25.6 (1994): 389-391.
2. Skipper, Ray, and Don DeStephano. "A Rapid Stain for *Campylobacter pylori* in Gastrointestinal Tissue Sections Using Diff-Quik." *The Journal of Histotechnology* 12.4 (1989): 303-304.
3. Modifications developed by Newcomer Supply Laboratory.
